Spinal neuroplasticity in chronic pain

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Introduction

Our body detects potentially noxious stimuli by means of nociceptors, specialized neurons which translate physical or chemical stimuli into electrical signals and transmit these to the central nervous system (CNS). The majority of these (primary) nociceptors are non-myelinated slowly conducting C fibers or thinly myelinated fast conducting Aδ fibers, the central endings of which end either in the dorsal horn of the spinal cord or in the trigeminal nucleus of the brainstem. The initial synaptic integration of nociceptive information occurs in these structures. Some of the spinal terminals of nociceptors directly stimulate projection neurons in the most dorsal layer of the spinal cord, the lamina I, while other nociceptor endings activate excitatory or inhibitory local interneurons in the same lamina as well as the underlying lamina II. Non-nociceptive mechanosensitive fibers that are activated by mild tactile stimuli end primarily in deep layers of the dorsal horn, where they come in contact with a different class of projection neurons, as well as with excitatory and inhibitory interneurons. In this way, the neuronal network of the dorsal horn integrates peripheral nociceptive and non-nociceptive stimuli with pro- and antinociceptive signals from pathways descending from the brainstem and midbrain into the spinal cord. The result of this spinal processing is then transmitted via several interconnections by lamina-I projection neurons in lamina I to higher CNS areas, where nociceptive and non-nociceptive stimuli are consciously perceived. Neuroplastic changes in this network represent an important mechanism of chronic pain.

Synaptic plasticity in lamina-I projection neurons

Lamina-I projection neurons in the dorsal horn play a crucial role in the perception of chronic inflammatory and neuropathic pain. The majority of these projection neurons are nocispecific neurons which, under physiological conditions, are activated exclusively by noxious stimuli. They carry neurokinin 1 (NK1) receptors for the neuropeptide substance P released from peptidergic C fibers. This latter characteristic has been used to destroy these spinal neurons in a targeted manner. Animals in which a conjugate comprising substance P and saporin was injected in the spinal cord were almost completely protected against inflammatory and neuropathic hyperalgesia [24]. Pursuing this idea, the plasticity of the synapses between primary nociceptors and these projection neurons have been closely investigated in recent years [13]. It could be demonstrated that intensive stimulation of these synapses triggers long-term potentiation (LTP). In particular, synapses with neurons that project to the periaqueductal gray (PAG), undergo LTP already with stimulation frequencies mimicking natural C fiber activity (**Fig. 1**).

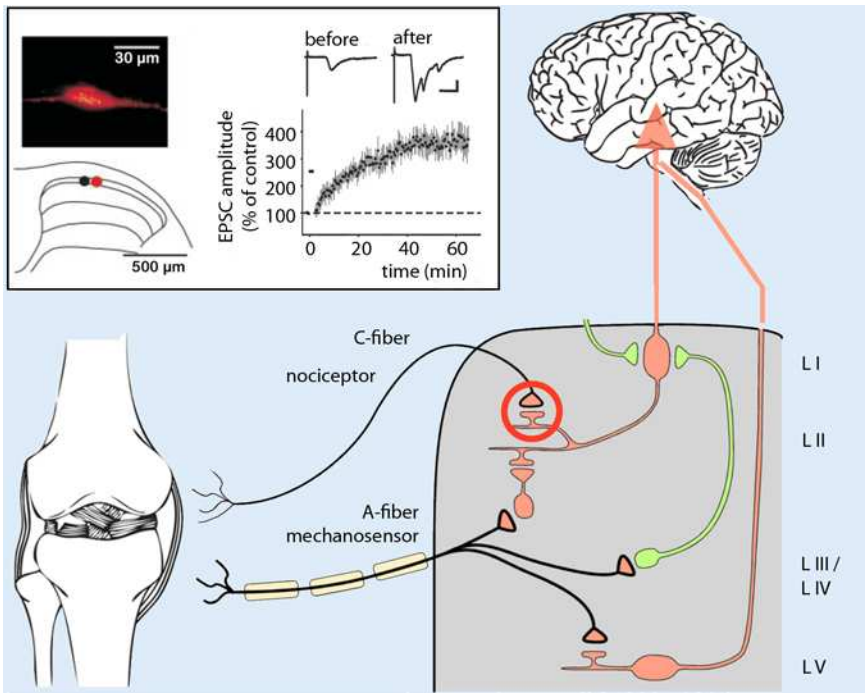

Fig. 1 ▲ Long-term potentiation at the synapse between primary nociceptors and nocispecific projection neurons in the lamina I. Intense stimulation of nociceptor endings leads to a persistent increase in synaptic transmission onto the projection neuron, as well as to hyperalgesia at the site of nociceptor stimulation. *LI–LV* indicate the various spinal laminae according to Rexed. (Modified from [13])

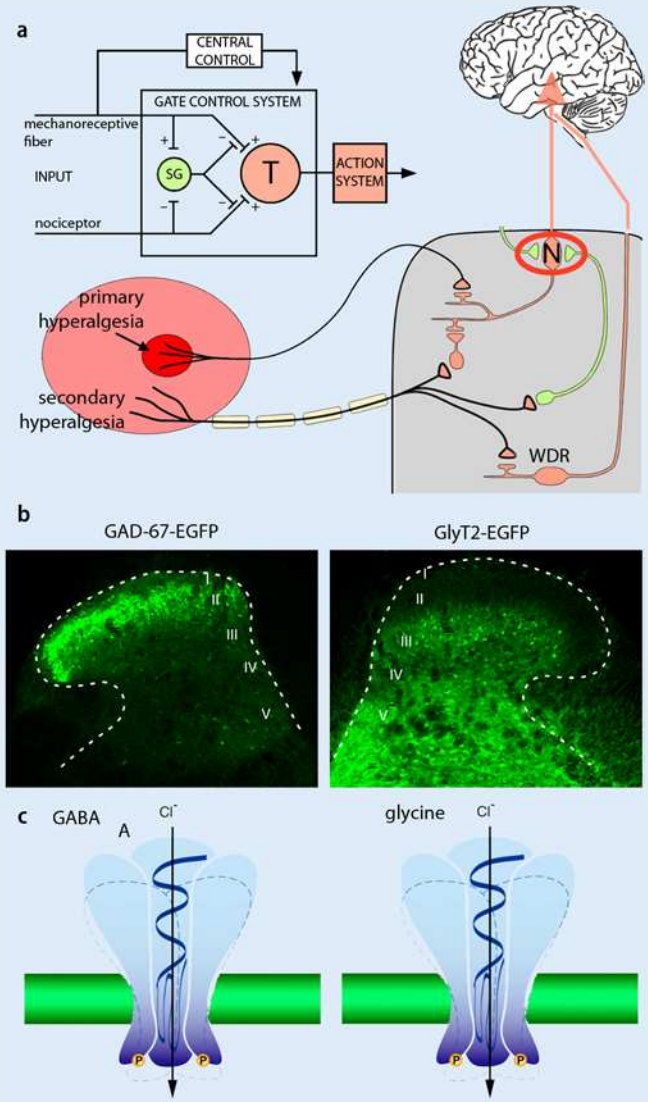

Fig. 2 ▲ **a** Primary and secondary hyperalgesia. In addition to sensitization at the site of nociceptor stimulation (primary hyperalgesia, *dark red area*), hypersensitivity to signals from non-nociceptive fibers also occurs in neighboring unconditioned areas (secondary hyperalgesia, *light red area*). Under physiological conditions, nociceptor signals are transmitted via nocispecific neurons (N) to supraspinal centers, while non-nociceptive signals are transmitted via wide dynamic range neurons in the deep dorsal horn. *Left* "Gate control" theory of pain (modified according to [18]). Inhibitory interneurons in the superficial dorsal horn, the substantia gelatinosa (*SG*), determine whether signals are transmitted through the spinal transmission system (*T*) to the brain. The activity of these inhibitory interneurons is inversely regulated by nociceptors and non-nociceptive fibers. **b** GABAergic and glycinergic interneurons, visualized here by cell type-specific expression of enhanced green fluorescent protein (*EGFP*), are abundant in the superficial dorsal horn. **c** Both transmitters open chloride channels ($GABA_A$ and strychnine-sensitive glycine receptors), which inhibit the activation of postsynaptic neurons

Inhibitory interneurons in spinal control of nociception

Although homosynaptic plasticity at C-fiber synapses is able to explain increased pain perception at the site of nociceptor stimulation (i.e., primary hyperalgesia), it is not able to simply explain why sensitization to signals from unconditioned inputs also occurs. Indeed, local C-fiber stimulation—experimentally inducible by, e.g., injection of capsaicin, a selective activator of nociceptors—also leads to hypersensitivity in unconditioned neighboring tissue and to pain originating from stimulation of non-nociceptive (capsaicin-insensitive) fibers (secondary hyperalgesia, ◘ **Fig. 2**). Interestingly, this secondary hyperplasia involves exclusively mechanical stimuli, while thermal sensitivity remains unaltered. This mechanical hypersensitivity is not based on the sensitization of peripheral nerve fibers, but rather is the result of altered central processing of sensory signals. Thus this secondary hyperplasia resembles heterosynaptic plasticity: intense stimulation of nociceptive C fibers leads to the sensitization of the body to signals from other unconditioned fibers. Detailed analysis has shown that, at least in part, these fibers are low-threshold mechanosensitive fibers, i.e., not nociceptors. Sensitization to signals from these fibers leads to the painful perception of normally painless, mild tactile stimuli, called allodynia. Neurophysiologically, this phenomenon is explained by the fact that signals from non-nociceptive fibers lead to the excitation of pain-signaling neurons. This unphysiological excitation of normally nocispecific projection neurons could be the result of generally increased projection neuron excitability. Alternatively, secondary hyperalgesia and allodynia could also be the result of reduced inhibitory control in the dorsal horn. A popular model to explain allodynia assumes the existence of two normally separate pathways for the spinal transmission of nociceptive and non-nociceptive stimuli. While nocispecific neurons are excited exclusively by nociceptive fibers (marked with "N" in ◘ **Fig. 2**), so-called wide dynamic range (WDR) neurons are excited by both nociceptive and non-nociceptive fibers. GABAergic and glycinergic neurons keep both pathways functionally separate in vivo. A critical role of this kind played by inhibitory interneurons in the superficial dorsal horn was already proposed more than 45 years in the "gate control" theory of pain [18] (◘ **Fig. 2a**). Indeed, inhibitory γ-aminobutyric acid-releasing (GABAergic) and glycinergic neurons are densely packed in the superficial dorsal horn (◘ **Fig. 2b**). Both neu-

rotransmitters inhibit the excitability of spinal neurons by opening chloride channels (**Fig. 2c**). In vivo pharmacological blockade of spinal glycine or $GABA_A$ receptors leads to symptoms similar to secondary hyperalgesia [27]. On a cellular level reduced inhibition demasks polysynaptic signals from non-nociceptive fibers occurs in previously nocispecific neurons [3, 29]. These findings show that non-nociceptive fibers are connected with normally nocispecific neurons by excitatory interneurons, and that these connections are masked by inhibitory neurons under physiological conditions. Moreover, GABA and glycine receptor blockade increases the response of lamina I neurons to C-fiber stimulation and induces spontaneous epilepsy-like discharge patterns in these neurons. In the intact organism, such cellular changes lead to corresponding sensory changes. Injecting $GABA_A$ or glycine receptor blockers induces hypersensitivity to noxious stimuli (hyperalgesia), nociceptive reactions following stimulation of non-nociceptive fibers (allodynia), and behavioral changes suggestive of spontaneous pain. Reducing GABAergic or glycinergic inhibition thus recapitulates important characteristics of pathological pain.

Endogenous mechanisms of disinhibition

Investigations carried out in recent years by various groups have shown that loss of spinal inhibitory control subsequent to peripheral inflammation, or neuropathies, or following intensive stimulation of nociceptors manifests itself endogenously.

Disinhibition in inflammation

In the context of inflammatory reactions, increased production of pronociceptive prostaglandins, in particular of prostaglandin E2 (PGE_2), is seen in peripheral inflamed tissue, but also in the CNS. Induction of cyclooxygenase-2 (COX-2), a key enzyme in prostaglandin synthesis, is a key process. On a cellular level, PGE_2 production results in facilitated transmission of signals from sensory afferents to second order neurons in the superficial dorsal [20], in direct depolarization of neurons in the deep dorsal horn [2], as well as in specific inhibition of glycine receptors in the superficial layers of the dorsal horn, i.e., in the central nociceptive innervation territories [34] (**Fig. 3**). PGE_2 produced in the spinal cord activates PGE_2 receptors of the EP2 subtype, leading to increased production of cAMP as well as protein kinase A stimulation [1]. This activation leads in turn to phosphorylation and inhibition of a particular isoform of glycine receptors containing the α3 subunit (GlyRα3 receptors) [9]. The contribution of this process to pain sensitization in the context of various pathologies has been investigated with the help of genetically manipulated mice lacking individual elements of this transduction cascade. EP2 receptor- and GlyRα3-deficient mice demonstrated an almost complete absence of nociceptive sensitization following spinal PGE_2 injection. Moreover, they recover far more rapidly from inflammation-induced pain sensitization than wild-type mice [1, 9, 33]. Further findings support the relevance of this prostaglandin-mediated disinhibition in inflammation-induced pain sensitization. Thus, mice lacking the catalytic subunit of neuronal protein kinase A also showed decreased pain sensitization by spinally injected PGE_2 [16]. Conditional COX-2-deficient mice lacking COX-2 specifically in the nervous system did not develop mechanical pain sensitization following peripheral inflammation [31]. Interestingly, EP2- and GlyRα3-deficient mice showed no changes in pain reactions in a series of other pain models. In particular, pain sensitization remained unchanged following peripheral nerve injury and chemical stimulation of nociceptors using capsaicin or formalin [10, 12].

Disinhibition following nociceptor stimulation

Loss of inhibitory spinal pain control also plays an important role in neuropathic pain and in pure activity-related forms of pain sensitization. As discussed above, intense stimulation of nociceptors causes not only nociceptive sensitization at the site of stimulation (primary hyperalgesia), but also in surrounding non-stimulated areas (secondary hyperalgesia, [19]).

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Keywords
Spinal cord · Pain · GABA · Glycine · Somatosensory processing

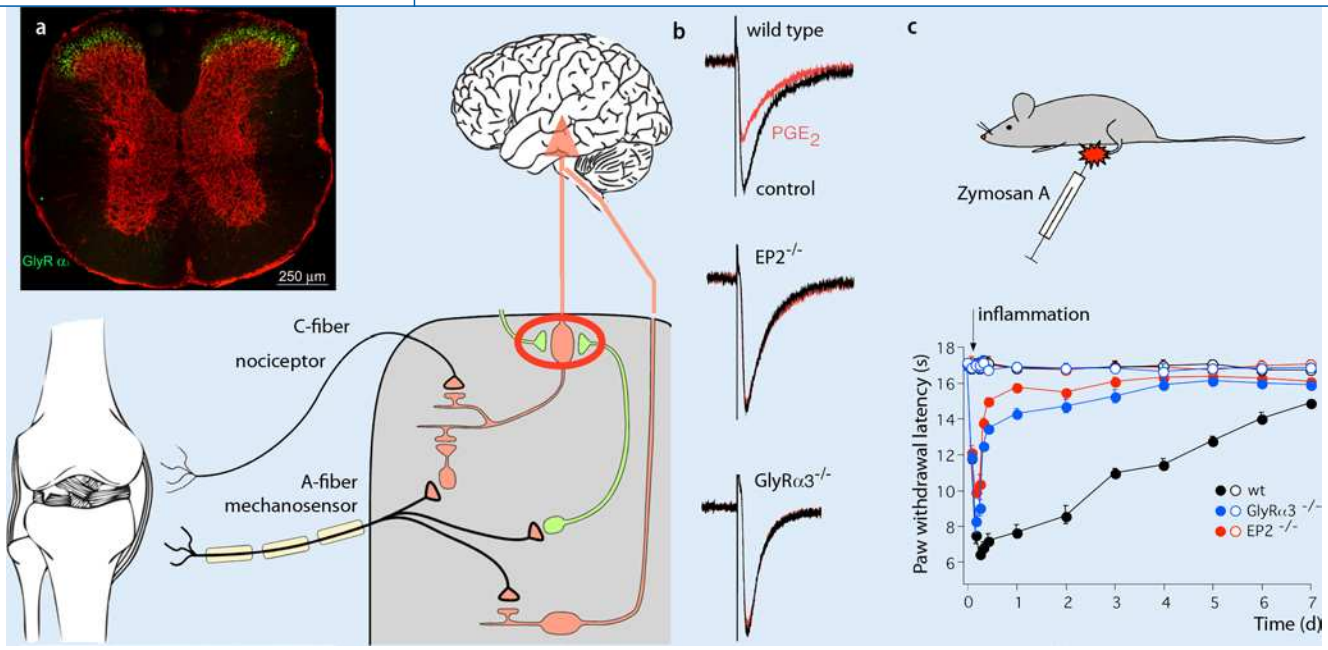

Fig. 3 ▲ Spinal disinhibition as a result of peripheral inflammation. **a** Strychnine-sensitive (inhibitory) glycine receptors are abundant in the dorsal horn. Those containing the α3 subunit are specifically concentrated in the superficial layers where nociceptor endings also lie. Activation of EP2 receptors by PGE_2 inhibits this glycine receptor subtype specifically via protein kinase A-dependent phosphorylation. **b** In mice lacking the EP2 receptor or Glyα3 subunit, no reduction in glycinergic inhibition by PGE_2 takes place. **c** These mice recover much faster from inflammatory hyperalgesia than wild-type mice. (**a,b** Modified according to [9]; **c** modified according to [33])

If one assumes that this activity- and C fiber-dependent form of secondary hyperalgesia also depends on disinhibition, intense spinal glutamate release from nociceptors should result in reduced synaptic inhibition by glycine or GABA. Heterosynaptic plasticity of this kind requires the existence of a diffusible messenger that transmits the information from nociceptive excitatory synapses to inhibitory synapses. Endocannabinoids and CB1 cannabinoid receptors couple intense glutamatergic excitation to diminished inhibition in numerous CNS regions [5]. The activation of CB1 receptors leads to reduced glycine and GABA release also in the dorsal horn of the spinal cord (**Fig. 4**). Mice lacking CB1 receptors, either globally or specifically from inhibitory dorsal horn neurons, are largely protected from capsaicin-induced mechanical pain sensitization [25]. Spinally injected CB1 receptor antagonists or group I metabotropic glutamate receptor antagonists can reverse secondary hyperalgesia in animal models. The pronociceptive effect of endocannabinoids produced in the spinal cord appears surprising at first glance given the many reports on the analgesic action of cannabinoids. However, it must be borne in mind that the reduced pain sensitivity in CB1 receptor-deficient mice and the analgesic action of CB1 receptor antagonists is limited to activity-dependent pain sensitization and has not been observed in inflammatory or neuropathic pain models. This is supported by findings from acute human pain models [15, 22], as well as from clinical studies in postoperative pain patients. In these settings, cannabinoids not only failed to show an analgesic effect, but instead resulted in increased pain. The fact that CB1 receptor agonists relieve pain in chronic pain patients could therefore mean that purely activity-dependent mechanisms of secondary hyperalgesia play only a minor role in chronic pain.

Disinhibition following peripheral nerve injury

A third form of pathological pain sensitization occurs following damage to the peripheral or central nervous system. In an experimental setting, neuropathic pain is usually evoked through mechanical lesion (ligature or partial transsection) of the sciatic nerve. This elicits sensitization of the affected extremity to thermal and mechanical stimuli, persisting over several weeks. In recent years, it has been observed that microglial cells play a crucial role in neuropathic pain sensitization (**Fig. 5**). Following peripheral nerve lesion, these cells are recruited in a multistage process in the spinal innervation area of the injured nerve fibers. After neuronal damage, primary sensory fibers release the cytokine CCL2 (also known as macrophage chemoattractant protein-1, MCP-1), which binds to CCR2 receptors on microglial cells [28, 36]. Further microglial activation depends on purinergic signals, which arise from increased expression of ionotropic P2X4 receptors on microglial cells. In order to cause pain sensitization, microglial activation must lead ultimately to altered neuronal communication. This task is fulfilled by brain-derived neurotrophic factor (BDNF), the production and release of which is triggered by activation of P2X4 receptors, microglial calcium signals and subsequent activation of the p38 MAP kinase signaling pathway [8]. BDNF binds to neuronal trk-B receptors and leads to increased expression of the potassium-chloride exporter KCC2 [7]. KCC2 keeps the intracellular chloride concentration in neu-

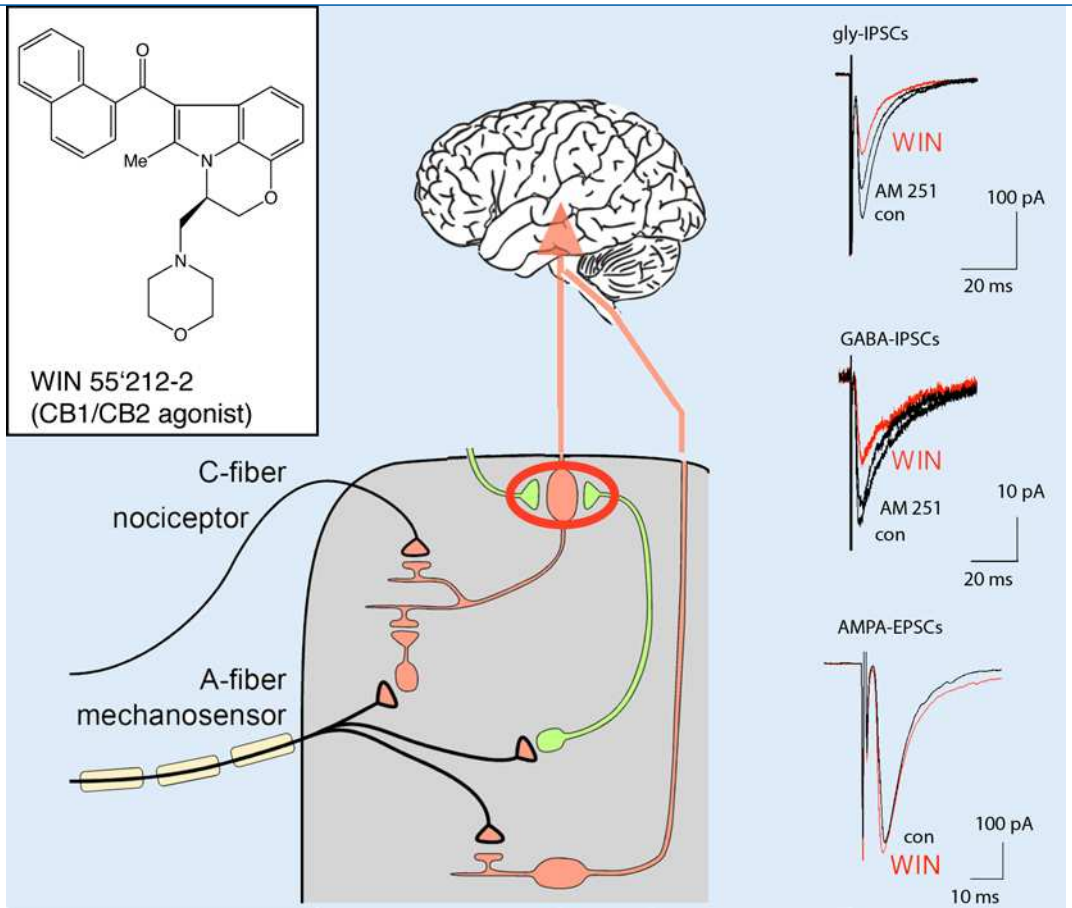

Fig. 4 ▶ Activation of cannabinoid CB1 receptors by the cannabinoid receptor agonists WIN 55,212-2 (*WIN*) results in reduced release of GABA and glycine, while glutamate release from excitatory spinal interneurons remains unaltered. Inhibition of GABA and glycine release by WIN 55,212-2 is reversed by the CB1 receptor antagonist AM251. Intense glutamate release from nociceptors induces the production and release of endocannabinoids and could thus link intense nociceptor stimulation to reduced synaptic inhibition. Endocannabinoids and CB1 receptors are thus able to function as mediators of secondary C fiber-transmitted hyperalgesia. (Modified according to [25])

rons low, thus enabling the hyperpolarizing action of GABA and glycine. Conversely, reduced KCC2 expression results in an increase in the intracellular chloride concentration and reduced inhibition by GABA and glycine. In extreme cases, GABAergic and glycinergic inhibition can even be reversed into depolarization and excitation. Neurons in the superficial dorsal horn, which have only a relatively low chloride extrusion capacity, appear to be most susceptible to these changes [6]. The question of whether GABA indeed acquires an excitatory effect or whether its inhibitory action is merely reduced is important for possible therapeutic implications. The majority of publications report that a local increase in GABAergic transmission mitigates neuropathic pain [14, 35]. It is difficult to reconcile this finding with an excitatory effect of GABA, suggesting rather that the inhibitory action of GABA and glycine is reduced but not changed into one of excitation. Alternatively, the analgesic effect of enhanced GABAergic transmission may result from an increase in shunting conductance.

Implications for the treatment of chronic pain

The findings discussed above demonstrate that pain pathologies of varying origin converge on a reduction of inhibitory pain control in the spinal cord. Pharmacologically increased GABAergic and glycinergic inhibition in the dorsal horn may therefore represent a new rational approach to the treatment of chronic pain, which should be equally effective irrespective of the contribution of inflammatory or neuropathic components. Indeed, increased synaptic inhibition by $GABA_A$ receptor modulators has a marked antihyperalgesic effect in inflammatory and neuropathic pain sensitization [35]. Investigations in $GABA_A$ receptor mutant mice show that these desired spinal effects are based on the activation of specific $GABA_A$ receptor subtypes containing the α2 and/or α3 subunit [14]. This specificity could prove crucial for the development of new pharmaceuticals, since the typical undesired effects of classic benzodiazepines (sedation, cognitive dysfunction, and addiction) require α1-$GABA_A$ receptor activation. At least in animal models, subtype-selective activators of $GABA_A$ receptors have shown the desired antihyperalgesic action, while undesired effects have been hitherto less pronounced [35].

In this context, increasing glycinergic inhibition pharmacologically could also prove to be very interesting. Currently, there are no compounds available which would do for glycine receptors what benzodiazepines do for $GABA_A$ receptors. However, glycine receptors still carry binding sites for allosteric modulators such as various cannabinoids [11]. Recent results even suggest that at least part of the analgesic action of cannabis (tetrahydrocannabinol, THC) is based on direct interaction with spinal glycine receptors [32].

A model of neuronal connectivity

A mechanistic understanding of the development of secondary hyperalgesia and

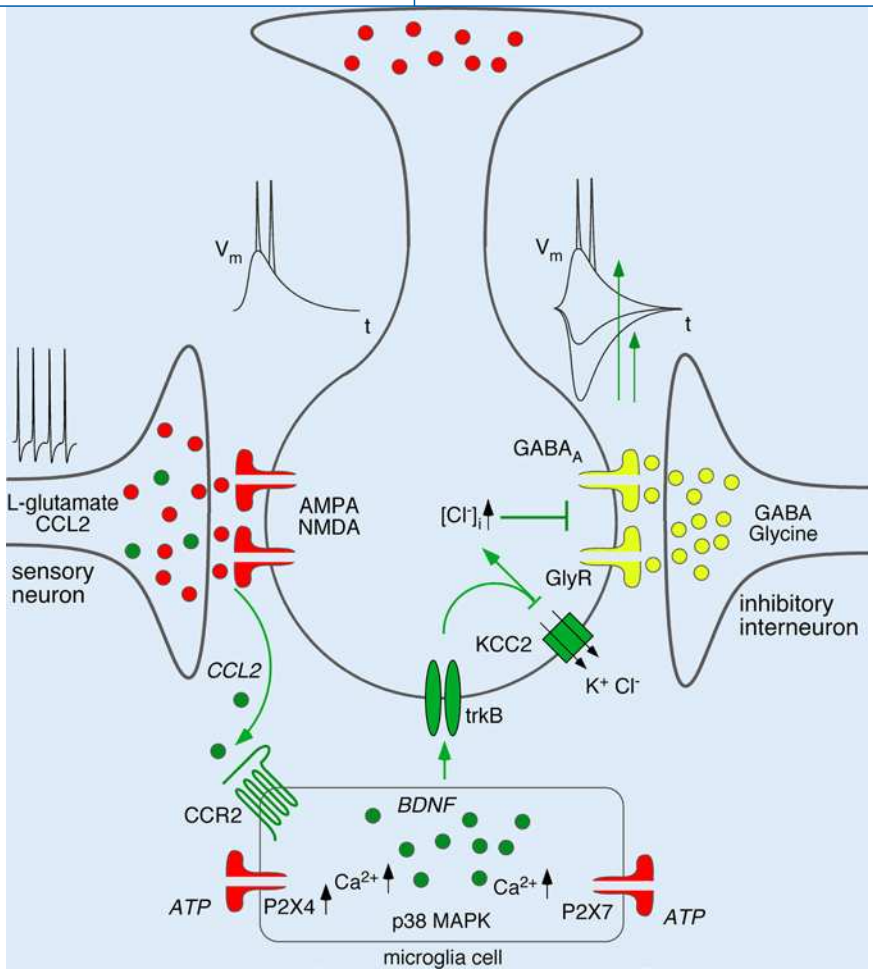

Fig. 5 ▲ The role of microglia in the development of hyperalgesia following peripheral nerve lesion. A central neuron is shown (*middle*) in the superficial dorsal horn, which receives synaptic signals from a damaged sensory fiber (*left*) and an inhibitory interneuron (*right*). Microglial cells (*bottom*) represent the functional connection between damaged nerve fibers and reduced synaptic inhibition. Following peripheral nerve lesion, the chemokine CCL2 is released from the endings of sensory nerve fibers. CCL2 recruits spinal microglia via activation of CCR2 receptors, additionally leading to increased expression of purinergic P2X2 receptors. Stimulation of the latter by ATP in turn induces the production and release of BDNF via a p38 MAP kinase-dependent process. BDNF then binds to trkB receptors on neurons in the dorsal horn, leading to reduced expression of the chloride exporter KCC2, which ultimately results in an increase in the intracellular chloride concentration and attenuated GABAergic and glycinergic inhibition. In this way, BDNF connects microglial activation with reduced synaptic inhibition in the dorsal horn

allodynia crucially depends on identifying the relevant sensory nerve fibers and spinal (inter-) neurons, as well as on accurate knowledge of their connectivity. Although we are still far from seeing the full picture, interesting observations have been made in sub-fields in recent years. Thus, a hitherto unknown fiber class could be identified which, when activated, leads to abnormal pain sensation in secondary hyperalgesic/allodynic areas. This class represents a subpopulation of non-myelinated, low-threshold mechanosensitive fibers characterized by the expression of a certain vesicular glutamate transporter (VGluT3) and the absence of typical characteristic peptidergic and non-peptidergic nociceptors [4]. The vast majority of these neurons bind neither isolectin B4 (IB4) nor antibodies to calcitonin gene-related peptide (CGRP), two classical markers of primary nociceptors. VGluT3-deficient mice showed markedly reduced secondary hyperalgesia following subcutaneous capsaicin injection, as well as reduced mechanical sensitization in the presence of inflammation, peripheral nerve lesion, or following cutaneous injury. These fibers terminate in the spinal cord in the lamina I and at the border between lamina II and III, in the immediate vicinity of protein kinase Cγ (PKCγ)-expressing neurons. These neurons are excitatory interneurons situated at the border between innervation regions of nociceptive and non-nociceptive fibers [23, 26]. Remarkably, PKCγ-deficient mice demonstrated strongly reduced hyperalgesia following peripheral nerve lesion and inflammation [17]. Moreover, blockade of PKCγ also prevents glycine receptor blockade-induced mechanical hyperalgesia. Experiments in which neuronal activation was demonstrated by *c-fos* expression also showed that PKCγ-positive neurons are activated by non-nociceptive but not by nociceptive fibers. Although not unequivocally proven to date, it is likely that PKCγ-positive neurons project via polysynaptic connections to normally nocispecific neurons. Interneurons with dendritic trees extending in a stellate formation (stellate cells) in the superficial posterior horn are possibly interconnected between VGlut3-positive fibers and PKCγ-positive neurons. The nature of the inhibitory neurons controlling this circuit is even less well understood. Functional neurobiological methods developed recently, such as targeted stimulation of defined neuronal populations by light-activated ion channels (channel rhodopsins) or their targeted destruction using cell-specific toxins, promise to provide new insights into this exciting field of research.

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